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Original scientific paper

DIFFERENT ASPECTS OF INHIBITION OF GROWTH AND PHOTOSYNTHESIS OF MAIZE (*Zea mays* L.) BY THE PHOSPHONATE HERBICIDE SULPHOSATE 1. ROOT MANIPULATION

Bogdan NIKOLIĆ[1,3], Goran DRINIĆ[2], Vladan JOVANOVIĆ[3], Vaskrsija JANJIĆ[3] and Sanja STOJAKOVIĆ[1]

[1]Institute of Plant Protection and Environment, Belgrade, Serbia
[2]Maize Research Institute Zemun-Polje, Belgrade, Serbia
[3]Institute of Pesticides and Environmental Protection, Belgrade, Serbia

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Effects of the herbicide sulphosate on growth, accumulation and partitioning of dry weight and photosynthesis in maize plants subjected to source-sink manipulation at the root were studied. The findings indicate that growth and dry weight accumulation correlate significantly only with the dry mass ratio and/or volume of the root (RMR, Vr, respectively), while a significant negative correlation was found with stem mass ratio (SMR) and generally with leaf mass ratio (LMR), which reflects an irregular distribution of carbohydrate metabolism in maize plants. As the root is where cytokinins, the plant hormons essential for maintaining photosynthetic structures, are synthesized, we assumed that the root status under stress caused by the herbicide sulphosate could be one of the factors of stability/sensitivity of photosynthesis/photosynthetic structures in plants exposed to this herbicide.

Corresponding author: Bogdan Nikolić, Institute of Plant Protection and Environment, Teodora Drajzera 9, 11000 Belgrade, Serbia

INTRODUCTION

Phosphonate herbicides (e.g. glyphosate, sulfosate) primarily induce inhibition of the shikimate biosynthetic pathway as a first assumed mode of action of these herbicides (1). Geiger et al. (7, 9) investigated the inhibition of photosynthesis and starch synthesis induced by the herbicide glyphosate early (1-2 h) after treatment. We reexamined those findings during inhibition of photosynthesis and growth of maize plants treated with sulphosate and their subjection to source-sink manipulation of the root.

MATERIALS AND METHODS

Maize (*Zea mays* L.; hyb. *ZPSC 704*) plants were grown under field conditions (PARmax>1500 μmol $m^{-2}s^{-1}$, variable photoperiod (15/9±1 h) and relatively stable temperature and humidity (28/22±4 ^{0}C, 50/60± 5%) over the July-August periods of 2002, 2003 and 2004. The plants were grown on organic compost for up to 4 weeks (nearly 5 fully grown leaves): one third of them in large pots (V=5 l; L plants) and two thirds in small pots (V=1 l). Three days before treatment, half of the plants growing in small pots (V=1 l) were transferred into large pots (RP plants), while the other half remained in small pots (V=1 l, S plants). L plants were not grown in 2003, which was a method of source-sink manipulation at the root. At the beginning of the trial, half the plants were treated with 10^{-2} mol of the sulphosate herbicide (syn. glyphosate-trimesium; product *Touchdown, Syngenta*, UK, 480 g/l a.i.) until run-off, and the other half (control plants) remained untreated. Samples were taken for an analysis of growth, dry weight partitioning, photosynthetic pigment content and root volume on the day of treatment, and the 4^{th} and 8^{th} post-treatment days. The parameters of growth and dry weight partitioning were defined and calculated according to POORTER AND GARNIER (1996) and DE GROOT ET AL. (2002).

Photosynthetic pigments were exposed to passive DMF extraction from samples at -20°C with sample absorbance (A_{664}, A_{647} and A_{480}) reading directly on the spectrophotometer, while Chl_a, Chl_b and total carotenoids (x+c) in leaf extract were calculated using Wellburn's (1994) formula. Root volume was determined using the Archimedes' Law. Chl_a fluorescence measurements (as well as sampling for RWC and photosynthetic pigment measurements) were done 20-25 cm below the tip of the youngest fully grown leaf on the day of treatment and on the 2^{nd}, 4^{th}, 6^{th} and 8^{th} post-treatment days using a *PAM 101/103* fluorometer in the first part of the photoperiod, following at least 3 hours of plant incubation in darkness. Parameters of Chl_a fluorescence were calculated according to MAXWELL AND JOHNSON (2000). For technical reasons, Chl_a flurescence measurements were performed within limits in 2003 and were not carried out at all in 2004. Statistical processing of the results acquired began by computing the means in *M Stat C* software (Michigan State University, USA). Statistical significance was tested by the analysis of variance (LSD test, the same software). Statistical significance of the differences found is marked with different letters and other symbols and stands for 5% statistical threshold. The significance of relationships between parameters was tested using correlation computation in the same software. The results of correlation computation are presented in tables with the results. The asterisk sign (*) marks significant (5%) and double askerisk (**) highly significant (1%) correlation.

RESULTS AND DISCUSSION

Dry weight accumulation (Fig. 1) of control plants in all plant groups (S, L and RP; 2002 trial) was significantly higher from the 4th day onwards than that of treated plants, which is concurrent with the differences in their growth. In this complex trial, we observed a low but highly significant correlation between ln DW and RMR, a negative highly significant correlation between RMR and SMR, and a negative significant correlation between LMR and all other parameters (Tab. 1).

Table 1. Correlation between parameters of accumulation and distribution of dry weight (2002 trial)

	ln DW	RMR	SMR	LMR
ln DW		0.291**	0.043	-0.252*
RMR			-0.424**	-0.253*
SMR				-0.253*
LMR				

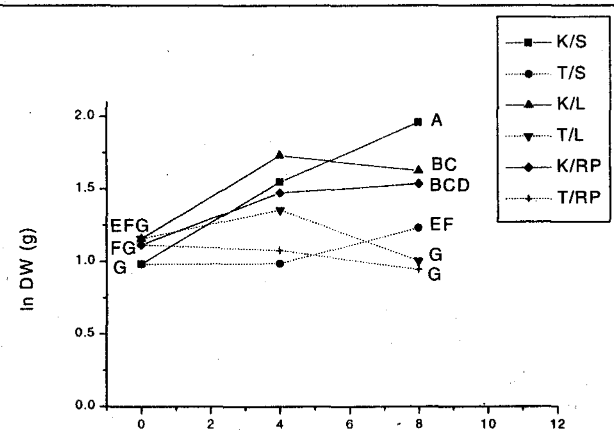

Figure 1. Accumulation of dry weight in control and treated (10^{-2} mol sulphosate) S, L and RP maize plants in an 8-day trial. The plants were grown under field conditions for 4 weeks. K/S, K/L, K/RP, T/S, T/L, T/RP: control or treated S plants (RGR_K =122.25 mg g^{-1} d^{-1}; RGR_T =31.12 mg g^{-1} d^{-1}), L plants (RGR_K =58.75 mg g^{-1} d^{-1}; RGR_T = -18.88 mg g^{-1} d^{-1}) and RP plants (RGR_K =52.88 mg g^{-1} d^{-1}; RGR_T = -15.50 mg g^{-1} d^{-1}) (2002 trial)

Beginning with the 6th day of trial, the electron transport rate (ETR) of control S plants was significantly higher than that of treated S plants (Fig. 2). Regarding the ETR parameter of L plants (Fig. 2), a significant difference was observed between control and treated plants as early as on the 2nd day of trial. In re-potted plants (RP plants), a significant inhibition of this photosynthetic parameter took place as early as on the 4th day (Fig. 2).

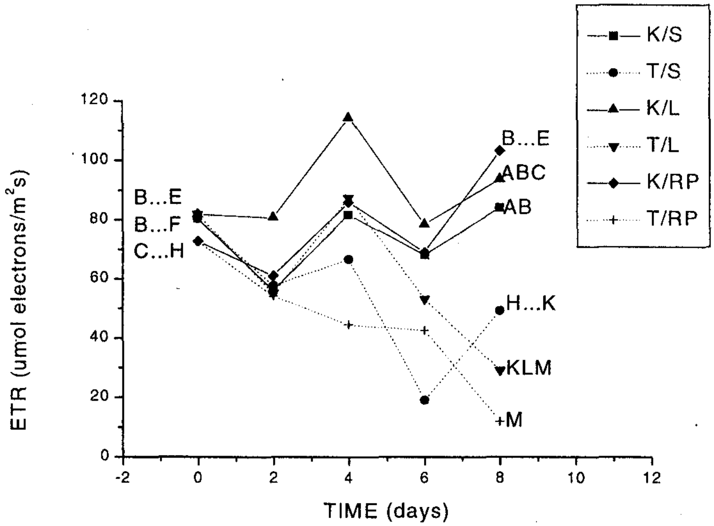

Figure 2. Changes in the photosynthesis parameter ETR of the 5th leaf of control and treated (10^{-2} mol sulphosate) S, L and RP maize plants in the 8-day trial. The plants were grown under field conditions for 4 weeks. K/S, K/L, K/RP, T/S, T/L, T/RP: control or treated S, L and RP plants (2002 trial)

The quantum efficiency parameters of photosystem II (Fv/Fm, Fv/F_0) were found to be in highly significant correlation mutually, as well as with the parameters qP, Φ PS II and ETR (Tab. 2). The parameters qP, Φ PS II and ETR were highly significantly correlated mutually, and each in highly significant negative correlation with the photoprotection NPQ parameter (Tab. 2). Φ PS II and Fv'/Fm' were also highly significantly correlated (Tab. 2). The parameters indicating the redox state of plastoquinone pool (qP, Φ PS II), as well as the Fv'/Fm' parameter, are more significantly correlated with the ETR of photosynthesis (Tab. 2) than the parameters of quantum efficiency of PS II (Fv/Fm. Fv/F_0). Suphosate-provoked inhibition of photosynthesis (ETR) is apparently cummulative in character, i.e. sulphosate affected several factors in the trial (Tab. 2). It means that the indicators of RC PS II state and redox state of the plastoquinone pool undergo different degrees of change under the activity of sulphosate (Tab. 2), which agrees with other reports (7, 9).

Table 2. Correlation between parameters of Chl_a fluorescence and photosynthesis (2002 trial)

	Fv/Fm	Fv/F$_0$	Φ PS II	qP	Fv'/Fm'	ETR	NPQ
Fv/Fm		0.901**	0.635**	0.705**	0.059	0.629**	0.081
Fv/F$_0$			0.659**	0.699**	0.084	0.659**	-0.004
Φ PS II				0.823**	0.512**	0.978**	-0.441**
qP					-0.010	0.802**	-0.441**
Fv'/Fm'						0.498**	-0.150
ETR							-0.417**
NPQ							

A significant inhibition of dry weight accumulation (Fig. 3) and growth was observed in treated S plants, although their growth in this trial (2003) was considerably slower than in the previous one (Fig. 1; 2002). In RP plants (Fig. 3), the accumulation of dry weight was moderate, and growth inhibition less pronounced than in S plants (but neverthless statistically significant). Although not prominent, the correlation between dry weight accumulation in this trial, and leaf mass ratio and root volume is highly significant (Tab. 3). The LMR parameter also had a highly significant negative correlation with RMR and especially with SMR, while the correlation between Vr and SMR was highly significantly negative, and RMR and SMR significantly negative (Tab. 3).

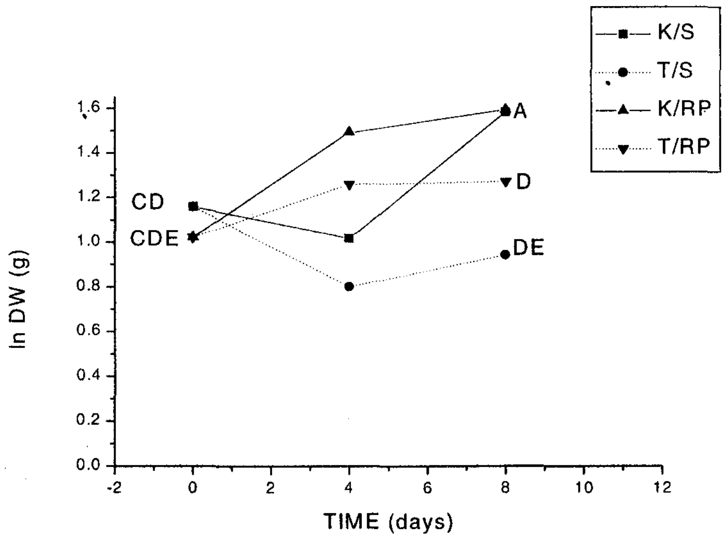

Figure 3. Dry weight accumulation in control and treated (10^{-2} mol sulphosate) S and RP maize plants in the 8-day trial. The plants were grown under field conditions for 4 weeks. K/S, K/RP, T/S, T/RP: control or treated S (RGR_K =52.88 mg g^{-1} d^{-1}; RGR_T= -27.00 mg g^{-1} d^{-1}) and RP (RGR_K =71.38 mg $g^{-1}d^{-1}$; RGR_T=31.12 mg g^{-1} d^{-1}) plants (2003 trial)

Table 3. Correlation between parameters of accumulation and allocation of dry weight and root volume (2003 trial)

	ln DW	RMR	SMR	LMR	Vr
ln DW		-0.207	-0.208	0.350**	0.400**
RMR			-0.313*	-0.459**	0.682**
SMR				-0.700**	-0.316**
LMR					-0.217
Vr					

However, the Fv/Fm values of control S plants (as an indicator of RC PS II activity) were rising, but without significant difference between control and treated plants (Tab. 4). On the 2nd day of trial alone a significant decrease in treated S plants was observed regarding this parameter, compared to control. In the final stage of the trial (6th day), sulphosate inhibited the photochemical activity of RC PS II of treated S plants (Tab. 4). According to available literature (2), the Fv/F_0 parameter (Tab. 4) is statistically more sensitive, so that significantly higher values were observed in control than in treated S plants, which indicates an increase in the photochemical activity of RC PS II.

Table 4. Chl_a fluorescence parameter of the 5th leaf of S maize plants grown under field conditions for 4 weeks. NM – not measured. (2003 trial)

Day/treatment (K/T)	Fv/Fm	$LSD_{0.05}$	$LSD_{0.01}$	Fv/F_0	$LSD_{0.05}$	$LSD_{0.01}$
0/K	0.762 BCD			3.212 FGH		
2/K	0.774 BCD			3.452 DEFG		
2/T	0.712 E			2.558 H		
4/K	0.791 ABCD			3.765 CDEF		
4/T	0.758 CD	0.045	0.060	3.672 CDEF	0.656	0.876
6/K	0.804 AB			4.106 ABCD		
6/T	NM			NM		
8/K	0.795 ABC			3.831 CDEF		
8/T	0.746 E			2.868 GH		

Table 5. Parameters of Chl_a fluorescence of the 5th leaf in RP maize plants grown under field conditions for 4 weeks. NM – not measured. (2003 trial)

Day/ treatment (K/T)	Fv/Fm	$LSD_{0.05}$	$LSD_{0.01}$	Fv/F_0	$LSD_{0.05}$	$LSD_{0.01}$
0/K	0.801 ABC			3.969 BCDE		
2/K	0.806 AB			4.118 ABC		
2/T	0.772 BCD			3.396 EFG		
4/K	0.804 AB			4.504 AB		
4/T	0.782 ABCD	0.045	0.060	3.635 CDEF	0.656	0.876
6/K	0.824 A			4.714 A		
6/T	NM			NM		
8/K	0.802 ABC			4.075 ABCD		
8/T	0.604 F			1.590 I		

The parameter Fv/Fm (Tab. 5) changed in a similar way as it did in S plants. Fv/F_0 was significantly inhibited in treated RP plants on the 8th day of this trial (Tab. 5). The two indicators of PS II quantum efficiency are highly significantly (0.901) correlated.

In the treated S plants, a significant decrease in Chl_a content (Fig. 4) is evident as early as on the 4th day, while the content of the Chl_a pigment (Fig. 4) was significantly lower in treated (than in control) RP plants no sooner than on the 8th day of trial.

All parameters showing the contents and relationships of photosynthetic pigments in this trial were highly significantly correlated (Table 6).

Table 6. Correlation of contents and ratios of photosynthetic pigmens (2003 trial)

	Chl_a	Chl_b	Chl_{a+b}	Chl_a/Chl_b	x+c	Chl_a/x+c
Chl_a		0.992**	1.000**	0.918**	0.881**	0.894**
Chl_b			0.995**	0.885**	0.900**	0.877**
Chl_{a+b}				0.912**	0.886**	0.891**
Chl_a/Chl_b					0.715**	0.952**
x+c						0.615**
Chl_a/x+c						

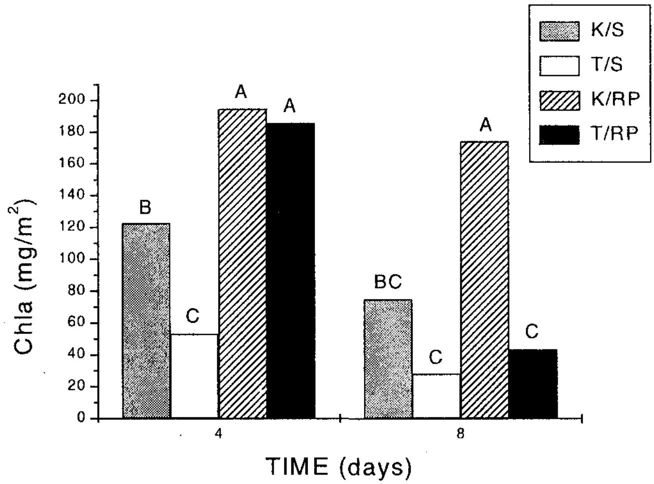

Figure 4. Changes in chlorophyll a (Chl_a) content in the 5^{th} leaf of control and treated (10^{-2} mol sulphosate) S and RP maize plants in the 8-day trial. The plants were grown under field conditions for 4 weeks. K/S, K/RP, T/S, T/RP: control or treated S and RP plants (2003 trial)

Dry weight accumulation (Fig. 5) in control S plants was prominent and significantly higher statistically than in treated plants, which indicates a significant inhibition of growth. The growth of S plants in this trial (2004) was comparable to that of corresponding plants in 2002.

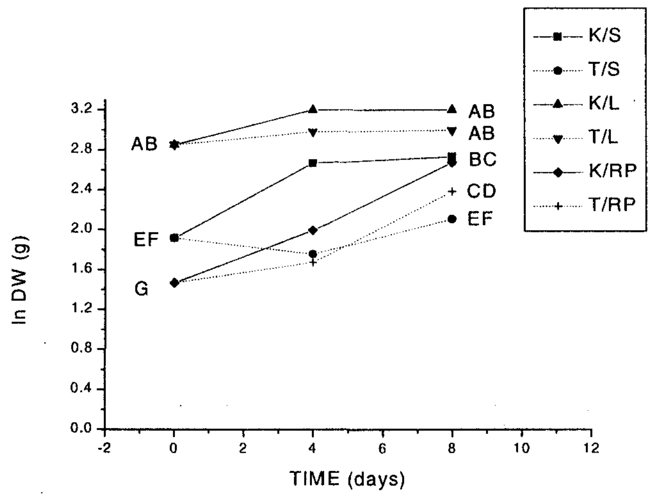

Figure 5. Accumulation of dry weight in control and treated (10^{-2} mol sulphosate) S, L and RP maize plants in the 8-day trial. The plants were grown under field conditions for 4 weeks. K/S, K/L, K/RP, T/S, T/L, T/RP: control or treated S (RGR_K =101.88 mg $g^{-1}d^{-1}$; RGR_T=23.88 mg $g^{-1}d^{-1}$), L (RGR_K=44.12 mg $g^{-1}d^{-1}$; RGR_T=18.62 mg $g^{-1}d^{-1}$) and RP (RGR_K=151.38 mg $g^{-1}d^{-1}$; RGR_T =114.50 mg $g^{-1}d^{-1}$) plants (2004 trial)

In L plants, dry weight accumulation was prominent but did not change significantly (Fig. 5) regardless of whether the plants had been treated with sulphosate or not. Consequently, plant growth was moderate, and sulphosate-caused inhibition of growth was not high. Comparing this trial (2004) with an earlier one (2002), it is evident that, despite a considerable difference in dry weight accumulation, the dynamics of growth and values of the RGR parameter of growth were similar.

In RP plants, dry weight accumulation was prominent (Fig. 5) and there were no significant differences between control and treated plants, so that growth was evident in both groups.

Dry weight accumulation is evidently highly correlated only with root volume (Vr). Vr correlates highly significantly with RMR, while a high negative correlation exists between Vr and LMR and a negative correlation between Vr and SMR (Tab. 7). RMR has a highly significant negative correlation both with SMR and LMR (Tab. 7).

Table 7. Correlation of the parameters of accumulation and distribution of dry weight and root volume (2004 trial)

	ln DW	RMR	SMR	LMR	Vr
ln DW		0.097	0.205	-0.073	0.715**
RMR			-0.578**	-0.672**	0.334**
SMR				0.023	-0.219*
LMR					-0.327**
Vr					

Our general conclusion is that dry weight accumulation and plant growth significantly correlate with root volume and/or root mass ratio (Vr, RMR), while correlation with SMR and (to considerable degree with) LMR is significantly negative, which reflects the irregular distribution of carbo-hydrate metabolism in maize plants (SETTER AND MELER, 1984). The root is known to be the site of synthesis of citokinins, i.e. plant hormons required for maintaining photosynthetic structures (PONS ET ALL., 2001). As our results (Fig. 1-5, Tab. 1-7) indicate an important role of the root under stress conditions provoked by the herbicide sulphosate, we believe that it could be a stabilizing factor in terms of photosynthesis/photosynthetic structures in plants exposed to the activity of that herbicide.

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RAZLIČITI ASPEKTI INHIBICIJE RASTENJA I FOTOSINTEZE KUKURUZA (*Zea mays* L.) UZROKOVANOG HERBICIDOM SULFOSATOM. 1. MANIPULACIJA STATUSOM KORENA

Bogdan NIKOLIĆ[1,3], Goran DRINIĆ[2], Vladan JOVANOVIĆ[3], Vaskrsija JANJIĆ[3] i Sanja STOJAKOVIĆ[1]

[1]Institut za zaštitu bilja i životnu sredinu, Beograd, Srbija
[2]Institut za kukuruz Zemun-Polje, Beograd, Srbija
[3]Institut za pesticide i zaštitu životne sredine, Beograd, Srbija

Izvod

U radu je razmatran uticaj herbicida sulfosata na rastenje, akumulaciju i preraspodelu suve mase, kao i fotosintezu biljaka kukuruza na kojima je vršena manipulacija "proizvođ-potrošač" odnosa na nivou korena. Nađeno je da akumulacija suve mase značajno koreliše samo sa zapreminom i/ili udeonom suvom masom korena (Vr, RMR), a negativno značajno sa udeonom suvom masom stabla (SMR) i (uglavnom) listova (LMR), što je u skladu sa neravnomernom preraspodelom ugljenohidratnog metabolizma kod biljaka kukuruza. Poznato je da je koren mesto sinteze citokinina, biljnih hormona neophodnih u održavanju fotosintetskih struktura. Pošto rezultati ukazuju na značaj statusa korena u uslovima stresa izazvanim herbicidom sulfosatom, smatramo da isti može biti jedan od faktora stabilnosti/ osetljivosti fotosinteze/ fotosintetskih struktura kod biljaka izloženih dejstvu pomenutog herbicida.

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